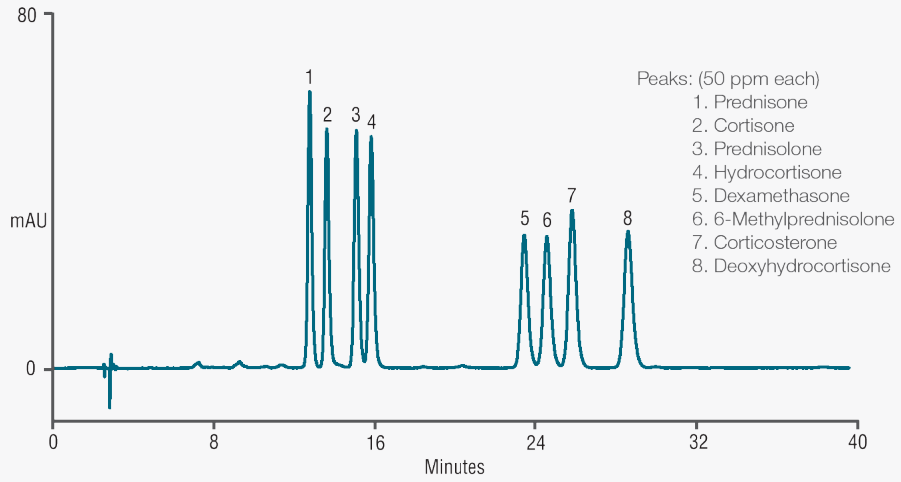
PRODUCT SPECIFICATIONS 22084

Acclaim Phenyl-1 column

Reversed-phase column with high aromatic selectivity

The Thermo Scientific™ Acclaim™ Phenyl-1 column ais designed to provide unique selectivity distinguished from other reversed-phase HPLC columns, resulting in superior separations for analytes that cannot be resolved well on typical alkyl phases (C18 and C8) or other phenyl-type columns.

Separation of glucocortocosteroid using the Acclaim Phenyl-1 column



Column chemistry

The Acclaim Phenyl-1 column is based on covalent modification of high-purity, spherical, porous silica particles, with a specially designed silane ligand bearing proprietary alkyl aromatic functionality. This novel column chemistry results in the following benefits:

- High aromatic selectivity
- High hydrophobic retention
- Unique and complementary selectivity
- Compatibility with highly aqueous mobile phase
- High efficiency and rugged packing

Chromatographic properties

Phenyl-type phases are usually superior in enhancing interaction with aromatic compounds. The unique chemistry of the Acclaim Phenyl-1 column further expands aromatic interaction for unique selectivity while maintaining sufficient hydrophobic interaction and aqueous compatibility.

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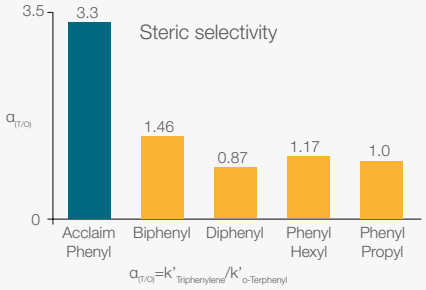
High aromatic selectivity

Triphenylene (T) and o-terphenyl (O) both contain the same carbon number, but in different shapes - the former planar, the latter propeller-shaped. Retention factor ratio between triphenylene (T) and *o*-terphenyl (O), $\alpha_{(T/O)} = k'_T/k'_O$ is often used as a descriptor to measure the shape selectivity of the stationary phase. However, when it comes to a phenyl-type column, this descriptor in fact measures the aromatic selectivity rather than shape selectivity of the phase. Enhanced aromatic selectivity is beneficial for applications in drug development and testing in which analytes commonly contain aromatic rings. As shown in Figure 1, the Acclaim Phenyl column shows the highest $\alpha_{(T/O)}$ value compared to other commercial phenyl-type stationary phases including biphenyl, diphenyl, C6-phenyl, and C3-phenyl phases.



Column	4.6 × 150 mm, 3 µm
Mobile phase	Acetonitrile/DI water v/v 80/20
Temperature	40 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV at 254 nm
Sample	0.5 mg/mL (each)
Probes	

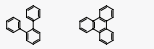
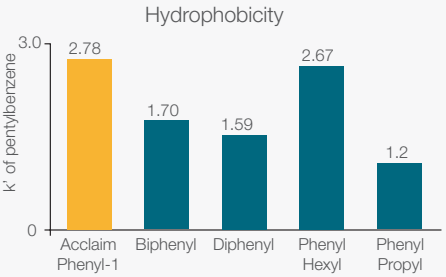


Figure 1. Aromatic selectivity comparison

High hydrophobic retention

Due to the incorporation of an alkyl chain into the stationary phase, the Acclaim Phenyl-1 column exhibits high hydrophobicity compared to other commercial phenyl-type phases, including biphenyl, diphenyl, C6-phenyl and C3-phenyl phases (Figure 2). This feature is highly desirable for retaining a broad range of analytes.

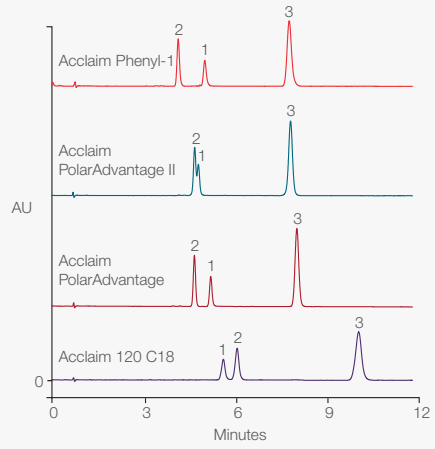


Column	4.6 × 150 mm, 3 µm
Mobile phase	Acetonitrile/DI water v/v 80/20
Temperature	40 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV at 254 nm
Sample	0.5 mg/mL (each)
Probe	

Figure 2. Hydrophobicity comparison

Unique and complementary selectivity

The National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 869a is useful for characterizing liquid chromatographic (LC) column selectivity for separation of polycyclic aromatic hydrocarbons (PAHs). This SRM is a mixture of three PAHs in acetonitrile: benzo[*a*]pyrene (BaP), 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN, alternate name, dibenzo[*g,p*] chrysene), and phenanthro[*3,4-c*] phenanthrene (PhPh) (see Figure 3 for structures). Depending on the elution order of the three components, column selectivity can be predicted for complex PAH mixtures. Figure 3 demonstrates the elution order of these three PAHs on the phenyl column and three other Acclaim reversed-phase columns: Thermo Scientific™ Acclaim™ 120 C18, Thermo Scientific™ Acclaim™ PolarAdvantage (sulfonamide-embedded), and Thermo Scientific™ Acclaim™ PolarAdvantage II (amide-embedded). It is clear that the phenyl column shows different and complementary selectivity.



Column	See traces
Dimensions	4.6 × 150 mm, 3 µm
Mobile phase	Acetonitrile/DI water v/v 85/15
Temperature	25 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV at 254 nm
Sample	NIST SRM 869a standard mix
Peaks	

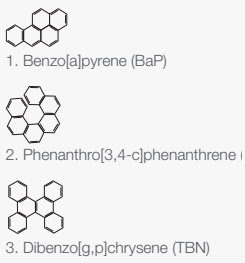


Figure 3. PAH selectivity comparison—separation of NIST SRM 869a

Compared to other commercial phenyl-type columns, the Acclaim Phenyl-1 column also exhibits unique selectivity (Figure 4). The high aromatic selectivity and affinity of the column retains aromatic compounds much more strongly, providing separation options for a much wider range of compounds.

Fully compatible with highly aqueous mobile phase

Most reversed-phase columns have high ligand densities, designed to improve the peak shape of basic analytes and stability at extreme pHs. However, dense surface coverage of silica particles with hydrocarbon chains often leads to inconsistent retention times in 100% aqueous conditions.

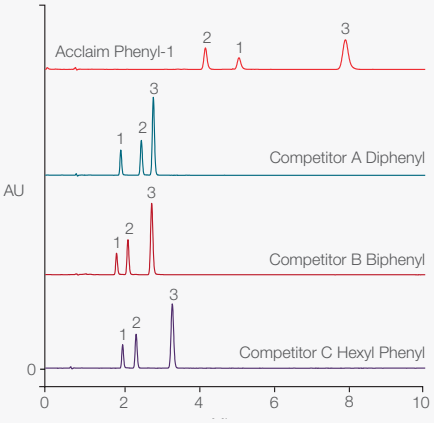
Although the low ligand density bonding is used to achieve better compatibility with 100% aqueous mobile phases, these phases provide poor peak shapes for bases and lower hydrolytic stability. As shown in Figure 5, the Acclaim Phenyl-1 column performs consistently well under 100% aqueous conditions—only negligible loss of retention was observed after 20 stop-flow cycles.

Applications

The Acclaim Phenyl-1 column is ideally suited for the analysis of aromatic analytes and has demonstrated its use in a wide variety of applications.

Glucocorticosteroids

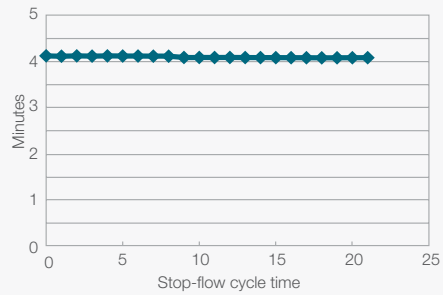
Glucocorticosteroids are a group of naturally occurring and synthetic hormones that moderate inflammation and other stress responses. All glucocorticosteroids are on the World Anti-Doping Agency's 2005 list of substances prohibited in competition when administered orally, rectally, intravenously, or intramuscularly. Separation of these substances has proven to be challenging. The unique chemistry of the Acclaim Phenyl column provides selectivity superior to conventional C18 columns for the separation of glucocorticosteroids. As shown in Figure 6, eight glucocorticosteroids are baseline resolved on a 3 × 250 mm Acclaim Phenyl-1 column using a methanol/water mobile phase system.



Column	See traces
Dimensions	4.6 × 150 mm, 3 µm
Mobile phase	Acetonitrile/DI water v/v 85/15
Temperature	25 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV at 254 nm
Sample	NIST SRM 869a standard mix
Peaks	

1. Benzo[a]pyrene (BaP)
2. Phenanthro[3,4-c]phenanthrene (PhPh)
3. Dibenzo[g,p]chrysene (TBN)

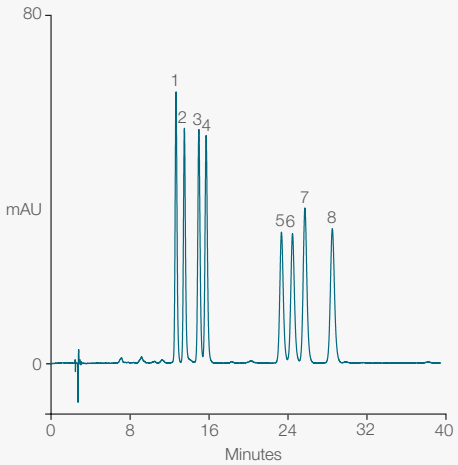
Figure 4. PAH selectivity comparison—separation of NIST SRM 869a



Column	Acclaim Phenyl-1, 3 µm
Dimensions	4.6 × 150 mm
Mobile phase	0.1% formic acid
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	2.5 µL
Detection	UV at 254 nm
Sample	0.1 mg/mL (each)
Probe	Thymine
Stop-flow protocol	

1. Test the column under above conditions
2. Stop the pump for 5 min (pump pressure drops to 0)
3. Resume the flow and condition the column under above conditions for 5 min
4. Inject the sample and run the test under the same conditions
5. Repeat 2 through 4

Figure 5. Compatibility with aqueous conditions



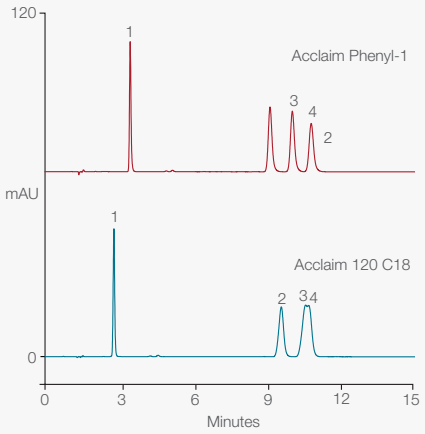
Column	Acclaim Phenyl-1, 3 µm
Dimension	3.0 × 250 mm
Mobile phase	Methanol/water v/v 46/54
Temperature:	40 °C
Flow rate	0.5 mL/min
Inj. volume	5 µL
Detection	UV at 254 nm
	(50 ppm each)
Peaks	1. Prednisone
	2. Cortisone
	3. Prednisolone
	4. Hydrocortisone
	5. Dexamethasone
	6. 6-Methylprednisolone
	7. Corticosterone
	8. Deoxyhydrocortisone

Figure 6. Separation of glucocorticosteroids

Estrogens

Estrogens are a group of steroid compounds, named for their importance in the estrous cycle, that function as the primary female sex hormone. Estrogens are used as part of some oral contraceptives and in estrogen-replacement therapy for postmenopausal women.

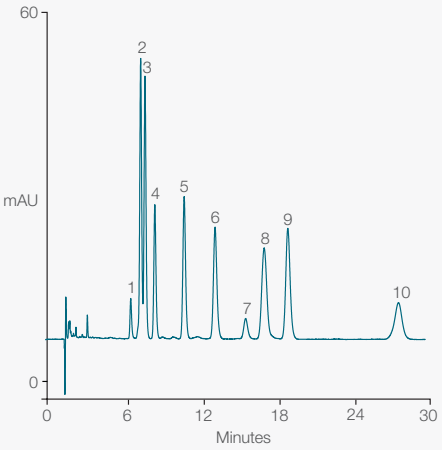
Three major naturally occurring estrogens in women are estrone (E1), estradiol (E2), and estriol (E3). Estradiol (E2) is the predominant form in nonpregnant females, estrone is produced during menopause, and estriol is the primary estrogen of pregnancy. Ethynylestradiol, a derivative of estradiol, is an orally bioactive estrogen used in almost all modern formulations of combined oral contraceptive pills. While the standard C18 column fails to separate these four compounds, the Acclaim Phenyl-1 column can baseline resolve them isocratically (Figure 7).



Columns	See traces
Dimensions	4.6 × 150 mm, 3 µm
Mobile phase	Acetonitrile/methanol/water v/v/v 15/40/45
Temperature	40 °C
Flow rate	1.2 mL/min
Inj. volume	5 µL
Detection	UV at 220 nm
Peaks	(100 ppm each)

1. Estriol (E3)
2. Estradiol (E2)
3. Estrone (E1)
4. Ethynylestradiol

Figure 7. Separation of estrogens



Column	Acclaim Phenyl-1, 3 µm
Dimension	3.0 × 150 mm
Mobile phase	Methanol/water v/v 90/10
Temperature	30 °C
Flow rate	0.5 mL/min
Inj. volume	2 µL
Detection	UV at 220 nm
Peaks	(100 ppm each) 1. Retinol acetate (vitamin A acetate) 2. Vitamin D2 3. Vitamin D3 4. δ-Tocopherol 5. γ-Tocopherol 6. α-Tocopherol (vitamin E) 7. Impurity (unknown) 8. Vitamin E acetate 9. Vitamin K_2 10. Vitamin K_1

Figure 8. Separation of fat-soluble vitamins

Fat-soluble vitamins

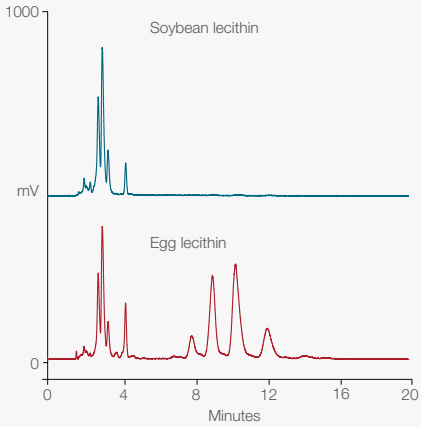
Along with water-soluble (B-complex and C) vitamins, fat-soluble (A, D, E, and K) vitamins are essential nutrients in small amounts for various roles in the human body. Vitamin A, also called retinol, has many functions in the body. In addition to helping the eyes adjust to light changes, vitamin A plays an important role in bone growth, tooth development, reproduction, cell division, and gene expression. In addition, the skin, eyes and mucous membranes of the mouth, nose, throat, and lungs depend on vitamin A to remain moist. Vitamin D plays a critical role in the body's use of calcium and phosphorous. It increases the amount of calcium absorbed from the small intestine and helps form and maintain bones. Children especially need adequate amounts of vitamin D to develop strong bones and healthy teeth. Vitamin E acts as an antioxidant, protecting vitamins A and C, red blood cells and essential fatty acids from destruction. Vitamin K plays an essential role in normal blood clotting and helps promote bone health.

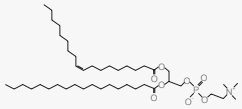
Unlike water-soluble vitamins that need regular replacement in the body, fat-soluble vitamins are stored in the liver and fatty tissues, and are eliminated at a much slower rate. At a result, they can pose a greater risk for toxicity than water-soluble vitamins when consumed in excess. On the other hand, some health problems may decrease the absorption of fat, and in turn, decrease the absorption of vitamins A, D, E, and K.

Like water-soluble vitamins, analysis of fat-soluble vitamins is an important and challenging assay for various products like pharmaceuticals, foods, beverages, and nutritional supplements. As shown in Figure 8, the Acclaim Phenyl-1 column provides excellent selectivity for separating vitamins A, D_2, D_3, K_1, K_2, as well as E and E acetate along with related substances, δ- and γ-tocopherols, under both gradient (not shown) and isocratic conditions.

Phospholipids

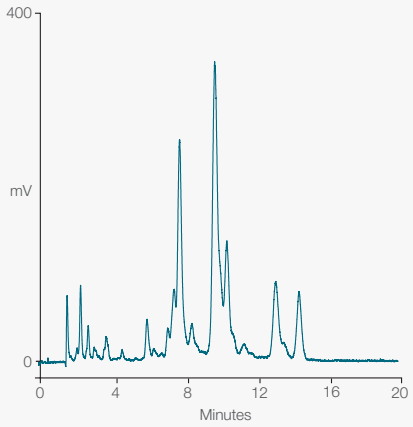
Lecithin is a generic term to designate the yellow-brownish fatty substances occurring in animal and plant tissues. Lecithin has emulsification and lubricant properties, and is a surfactant. Thus, it is widely used for applications in human food, animal feed, pharmaceuticals, paint, and other industrial applications. Phospholipids are a class of lipids and are a major component of all cell membranes as they can form lipid bilayers. Depending on the source, the composition of lecithin can vary. Figure 9 shows the profiles of lecithin from egg yolk and soybean obtained on an Acclaim Phenyl-1 column using a Corona *ultra* CAD detector. While both egg yolk and soybean contain phospholipids (e.g., phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol) as early eluting peaks (in 2 to 4 min range), it is indicated that egg yolk has a significant quantity of triglycerides as later eluting peaks (from 7 to 13 min). To obtain detailed information on phospholipids composition, a mobile phase containing higher aqueous content and less isopropanol is used to resolve major components of phospholipids in soybean lecithin (Figure 10).





Column	Acclaim Phenyl-1, 3 µm
Dimension	3.0 × 150 mm
Mobile phase	Acetonitrile/2-propanol/ammonium acetate (100 mM, pH 5.0) v/v/v 45/45/10
Temperature	25 °C
Flow rate	0.45 mL/min
Inj. volume	2 µL
Detection	Corona *ultra* (Gain = 100 pA; Filter = low; Nebulizer temp. = 25 °C)
Sample	Soybean lecithin (1 mg/mL in 2-propanol)
	Egg lecithin (2.5 mg/mL in 2-propanol)

Figure 9. Analysis of soybean and egg lecithin



Column	Acclaim Phenyl-1, 3 µm
Dimension	3.0 × 150 mm
Mobile phase	Acetonitrile/2-propanol/ammonium acetate (100 mM, pH 5.0) v/v/v 70/10/20
Temperature	25 °C
Flow rate	0.45 mL/min
Inj. volume	5 µL
Detection	Corona *ultra* (Gain = 100 pA; Filter = low; Nebulizer temp. = 25 °C)
Sample	Soybean lecithin (1 mg/mL in 2-propanol)

Figure 10. Phospholipids in soybean lecithin

Reproducible manufacturing

Each Acclaim column is manufactured to strict specifications to ensure column-to-column reproducibility.

Each column is individually tested and shipped with a qualification assurance report.

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Column specifications

Specifications	
Column chemistry	Proprietary alkyl phenyl
Silica substrate	Particle size: 3 µm Surface area: 300 m^2/g Pore size: 120 Å
pH range	2.0 to 8.0
Temperature limit	60 °C
Operating pressure (Max)	8500 psi for 150 mm (length) columns 8500 psi for 100 mm (length) columns
Operating flow rate (Max)	2.0 mL/min for 4.6 mm ID. columns 1.0 mL/min for 3.0 mm ID columns 0.5 mL/min for 2.1 mm ID columns

Ordering information

Column	Particle size (µm)	Format	Length (mm)	ID (mm)	Part number
Acclaim Phenyl-1	3.0	Analytical	150	4.6	071969
			150	3.0	071970
			100	3.0	074693
			150	2.1	071971
		Guard	10	4.6	071973
			10	3.0	071974

Acclaim Guard Holder ordering information

Guard holder	Part number
Thermo Scientific™ Acclaim™ Guard Cartridge Holder V-2	069580
Thermo Scientific™ Acclaim™ Guard Kit (Holder and coupler) V-2	069707
Guard to Analytical Column Coupler V-2	074188

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